REVISIÓN

Bronchoalveolar lavage studies of pulmonary surfactant

Patricia L. Haslam

Cell Biology Unit, National Heart & Lung Institute, Dovehouse Street, Londres SW3 6LY, Reino Unido.

Introduction

Pulmonary surfactant is a complex material, containing highly surface-active components, which spreads to cover the alveolar epithelial surfaces of the lungs[1]. Because of its surface-active properties, it plays an important role in lung function by reducing the surface tension of the alveolar epithelial lining fluid to counteract the forces which would otherwiese cause the alveoli to flood, and the lungs to collapse at low lung volumes when the intra-alveolar pressures are low compared with the transpulmonary pressures[2]. The main components of the pulmonary surfactant system are phospholipids which account for about 85% of the total components; and the main phospholipid component is dipalmitoylphosphatidylcholine (DPPC), which is also the main surface active component. In addition, the surfactant system contains about 8% proteins, 5% neutral lipids and 2% carbohydrates. In recent years, it has been shown that a number of the protein components are specific to the surfactant system and they, together with the main phospholipid components, are essential for the efficient functioning and turnover of surfactant components[3]. The main phospholipids and specific proteins of the surfactant system are synthesised and secreted by the type II alveolar lining cells. After synthesis, the phospholipids are stored within the cytoplasm of the type II cells in specialised organelles termed lamellar bodies, from which they are released onto the alveolar epithelial surfaces. The surfactant-specific proteins are transported separately to the cell surface in multivesicular bodies. The released phospholipids and proteins interact extracellularly in the aqueous hypophase of the alveolar epithelial lining fluid to form unusual lattice structures known as tubular myelin, and these structures aid the adsorption and spreading of a monolayer film of phospholipid onto the surface of the epithelial lining fluid. Because of their polar nature, the molecules of phospholipid align at the air-liquid interface with their hydrophilic head groups in the liquid and their hydrophobic fatty acid tails in the air space. The compression of these molecules during expiration generates an increasing film pressure which opposes, and reduces, the force of surface tension at the air-liquid interface. This minimises the pressure differences across the air-liquid interface as the alveoli change in size, consequently stabilising the alveoli and allowing them to remain open during the entire cycle of breathing. The cycles of contraction and expansion exhaust the surface phospholipid which is continuously expelled from the monolayer and recycled back to the type II cells for re-synthesis. Some of the exhausted phospholipid is also removed through phagocytosis by alveolar macrophages. Phospolipid recycling to type II cells is aided by the main surfactant-specific protein, surfactant apoprotein A (SP-A), which interacts with phospholipids and attaches to SP-A receptors on the surface of the type II cells[4]. Thus the synthesis and turnover of surfactant phospholipids and apoproteins is a highly dynamic process; and it is clear that any pathological event wich causes a marked defect in surfactant synthesis and/or turnover could have catastrophic consequences. This is dramatically demonstrated in the infant respiratory distress syndrome (IRDS), where defective production of surfactant in premature infants due to immaturity of type II cells at the time of birth can lead to acute respiratory failure because of inadequate alveolar inflation causing alveolar instability, flooding and collapse. The classic report of Avery & Mead in 1959 led to the recognition that pulmonary surfactant deficiency is the primary aetiologic factor in the pathogenesis of IRDS[5]. Numerous subsequent investigations have provided extensive information on the biochemical composition and biophysical function of the pulmonary surfactant system and on the nature of the defect in IRDS[1,2]. Various types of exogenous surfactant have been developed for use in the treatment of IRDS, and these are now of established therapeutic benefit. The consensus from many clinical trials is that both natural and synthetic surfactants can reduce mortality by up to 50% when used prophylactically in premature infants at risk of IRDS, and that natural surfactants can also reduce mortality by up to 40% when used in rescue therapy in infants with established IRDS[6].

Despite the extensive knowledge of the surfactant system gained in the paediatric field, much less is known about the surfactant system in adult lung diseases. There is, however, much current interest in the prospects for employing exogenous surfactant therapy in the adult respiratory distress syndrome (ARDS). This catastrophic acute lung injury can occur in asso-

Arch Bronconeumol 1994; 30:207-211

ciation with a wide range of risk factors; and it is characterised by widespread high permeability pulmonary oedema, massive atelectasis and respiratory failure with clinical and histological features resembling the IRDS[7]. Surfactant changes are not the primary aetiologic factor in ARDS, but they develop as a secondary factor during the course of the syndrome and are a most important contributory factor in the pathophysiology. The technique of bronchoalveolar lavage (BAL) facilitates studies of the surfactant system in adult lungs, and the aim of the remainder of this review is to summarise some of the current information gained using BAL to investigate normal human pulmonary surfactant in adult volunteers, and to study the compositional alterations which occur in ARDS and in other adult lung diseases. Evidence that the pulmonary surfactant system may play an important role in host defence, will also be summarised.

Normal pulmonary surfactant

Information on the composition of normal pulmonary surfactant has been obtained by investigating BAL samples from healthy adult volunteers. The BAL samples are spun at low speed ($\leq$ 300 g) for 10 minutes at 4 °C immediately after collection, to remove cells which might otherwise contaminate the surfactant components with cell-derived lipids and proteins. High centrifugation forces must not be used because, apart from inducing cell damage, these can also sediment and cause loss of aggregates of surfactant phospholipids and proteins in the form of tubular myelin. To evaluate the surfactant phospholipid composition, lipids are extracted from the cell-free BAL fluids using conventional methods, for example chloroform-methanol extraction, then the main phospholipid classes are separated and quantified by thin-layer chromatography or high-pressure liquid chromatography[1,2]. The results of such studies have shown that phosphatidylcholine (PC) accounts for on average 73% of the phospholipid in lavage samples from normal healthy non-smoking human volunteers, and that it is mostly in the form of saturated DPPC which is highly surface active. Phosphatidylglycerol (PG) is the second major phospholipid component accounting for on average 12% of the phospholipid, while the remainder include the minor components phosphatidylinositol (PI), phosphatidylethanolamine (PE), phosphatidylserine (PS), sphingomyelin (SM), cardiolipin, and lysophosphatidylcholine none of which account for an average of more than 3% of the total phospholipid. By contrast with the phospholipid composition of pulmonary surfactant, phospholipids in lung tissue and blood plasma contain lower proportions of PC (mean $<$ 50%), and much lower proportions of PG ($<$ 2%), but lung tissue contains higher proportions of PE (mean 17%) and both lung tissue and blood plasma contain higher proportions of SM (mean 11% and 24% respectively). In pathological situations, damage to lung tissue and leakage of plasma into the alveolar air spaces can contaminate the pulmonary surfactant system. This can result in abnormal elevations in PE and SM (as well as in proteins and other tissue or plasma-derived components) in the BAL samples. Interestingly, we have observed that BAL samples from apparently healthy normal volunteer cigarette smokers contain significantly higher proportions of PE and SM compared with non-smoking volunteers[8], indicating that smoking-related tissue damage is sufficient to have a measurable effect on the surfactant system. The functional consequences of such contamination in smokers has not yet been investigated.

There is much less information about the specific protein components of the pulmonary surfactant system, since these have only been discovered in relatively recent years[4]. However, four proteins have been identified to date including the hydrophilic glycoproteins surfactant apoprotein A (SP-A) and SP-D, and the hydrophobic apoproteins SP-B and SP-C. SP-A is the major surfactant apoprotein and it interacts with phospholipids[9], SP-B, SP-C and calcium ions to participate in the formation of tubular myelin. SP-B and SP-C play a further essential role in enhancing the adsorption and spreading of the monolayer of phospholipid at the air-liquid interface of the lung lining fluid; and SP-B also promotes the squeezing out of impurities from the lipid monolayer[10]. SP-A then regulates phospholipid recycling to type II cells for resynthesis and regeneration[4]. There is also evidence that SP-A plays a role in host defence by enhancing the phagocytosis and killing of micro-organisms by alveolar macrophages; and recent evidence suggests that SP-D may play a similar role in host defence. Currently, little is known about the levels of surfactant apoproteins in normal BAL samples and any changes which might occur in human lung diseases. This is an important field of current research, and it is probable that synthetic surfactants now being developed containing recombinant surfactant apoproteins in addition to phospholipids, may prove an important advance because they more closely mimic the natural product.

Adult respiratory distress syndrome

Catastrophic acute lung injury resulting in ARDS can occur in previously healthy individuals after exposure to a wide variety of risk factors, including sepsis syndrome, infectious pneumonias, aspiration pneumonia, cardiopulmonary bypass, smoke inhalation, multiple blood transfusions, disseminated intravascular coagulation, trauma or multiple fractures[7]. The initial insult, by mechanisms not fully understood, leads to massive pulmonary oedema as a result of increased vascular capillary permeability to plasma proteins due to damage to the alveolar endothelium and epithelium. Patients develop life-threatening acute respiratory failure which in many cases progresses to multi-organ failure and death. The syndrome was first described by Ashbaugh et al in 1967[11], and the mortality rate still remains at 60-70% despite improvements in mechanical ventilation. The mechanisms

which initiate the syndrome are varied but they all involve processes which damage the pulmonary vascular capillaries. In many cases, this appears to be the consequence of the activation of circulating neutrophils resulting in their increased accumulation within the pulmonary capillaries and extravasation into the interstitium and air spaces. The neutrophils exhibit enhanced surface expression of adhesion molecules (including the CD11/18 group of molecules and L-selectin), and enhanced levels of adhesion molecules (including the ICAMS and P- and E-selectins) are also thought to be expressed on the vascular endothelial cells. Activated neutrophils adherent to the endothelium are then thought to cause endothelial damage by releasing toxic oxygen radicals, proteolytic enzymes and other potentially damaging mediators. Leakage of plasma components through the damaged endothelium into the alveolar spaces then contaminates the pulmonary surfactant system sufficiently to result in its dysfunction. Alveolar macrophages become activated as a consequence of the inflammatory response, and then contribute to amplify endothelial and epithelial damage through the release of oxidants and inflammatory mediators, including the cytokines interleukin-1 and tumour necrosis factor-α, and interleukin-8, which are thought to enhance the expression of adhesion molecules promotes the recruitment of neutrophils. BAL samples from patients with established ARDS contain strikingly increased numbers of neutrophils often accounting for more than 70% of the total BAL cells[12].

Measurement of total protein and albumin levels in the BAL samples of ARDS patients provides a useful quantitative and qualitative indication of the extent of contamination of the surfactant system by proteins derived either from plasma leakage or damaged tissue components. To allow for the problem of uncontrolled dilution of components during the BAL procedure, we recommend expressing the results of total protein and albumin measurements as ratios: total phospholipid (PL) and: PC. These rations are significantly increased in patients with established ARDS compared to healthy controls. The majority of patients with ARDS also have evidence of defective surfactant production, suggesting type II cell damage. This is indicated by significant decreases in the proportion (relative to the total BAL phospholipid) composed of the major surfactant phospholipid PC, and in the levels and proportions of the second major phospholipid component of the surfactant system PG. Proportions of SM are also increased in BAL in ARDS giving an additional indication of contamination of surfactant from tissue or plasma.

Currently, there is much interest in the prospects for surfactant therapy in ARDS; and a clinical trial of the artificial surfactant 'Exosurf' (Wellcome Foundation), introduced by nebuliser over the first five days in patients with sepsis-related ARDS, is in progress in the USA. The results are still awaited. However, more information is needed about the exact nature of the surfactant alterations at different stages in the development of ARDS and in relation to different risk factors to better define the optimal rationale for therapy. Serial BAL studies may provide such information, and are in progress at our own and other centres. There has been one detailed report of serial BAL studies in patients with ARDS associated with trauma[13] showing that total protein levels in BAL were strikingly increased throughout the first week of the syndrome in patients with severe ARDS, then tended to fall during the second week buet were still above normal control levels at the end of 14 day follow-up. By contrast, proportions of the major surfactant phospholipid PC were only moderately decreased during the first week but fell progressively and were strikingly decreased by the second week. Thus in patients with trauma-associated ARDS, surfactant therapy at an early stage of the syndrome would need to overcome surfactant deficiency due mainly to protein contamination but therapy at later stages would need to overcome deficiency due to both protein contamination and defective production of surfactant components. There is no reported information on attempts to treat late-stage ARDS with exogenous surfactant, but we have recently had the opportunity to investigate four patients treated in the Adult Intensive Care Department of the Royal Brompton Hospital with 'Artificial Lung Expanding Compound (ALEC)' (Britannia Pharmaceuticals), 'ALEC' is a mixture of 70% DPPC and 30% unsaturated PG, and it was introduced via intratracheal instillation in a total dose of 3.2 g. The treatment was of no sustained objective clinical benefit but BAL samples before and 24 hours after therapy showed that it achieved a measurable supplementation of in vivo PC and PG levels and proportions[14]. Our findings further suggest that therapeutic formulations to achieve greater supplementation of PC may be required. Combined treatment with anti-inflammatory drugs may also be indicated since we also observed that surfactant therapy did not result in any significant suppression of neutrophils in BAL. There is little information on levels of surfactant-specific proteins in patients with ARDS, but Gregory et al[15] have recently reported that both SP-A and SP-B levels are significantly decreased in BAL and that SP-A levels are also decreased in patients at risk. This suggests that therapeutic surfactants containing surfactant-associated proteins in addition to phospholipids may be of greater potential benefit in the treatment of ARDS than preparations containing mainly phospholipid.

Regarding anti-inflammatory therapy in patients with ARDS, many clinical trials of corticosteroids have been conducted with the aim of suppressing the release of inflammatory mediators by neutrophils[16]. Most of these trials have concluded that corticosteroids are of no therapeutic benefit either for prophylaxis in patients at risk (mainly sepsis syndrome) or for early-stage treatment of ARDS. However, there have been a number of recent case reports indicating that corticosteroids can be of value in those patients with late-stage ARDS who develop the complication of

pulmonary fibrosis[17]. We have reported one such case in whom both BAL neutrophil counts and phospholipid levels and proportions of PC and PG normalised in association with a striking clinical response[18]. This observation suggests that the beneficial effects of corticosteroids may be due to not only the antiinflammatory effects but also to the known ability of corticosteroids to stimulate the secretion of surfactant phospholipids by type II alveolar epithelial cells, either directly or by stimulating fibroblasts to produce a 'fibroblast pneumocyte factor' which can enhance surfactant synthesis[19].

Chronic fibrosing lung diseases

In view of the beneficial effect of corticosteroid therapy on the fibrotic stage of ARDS, it is of interest that we have observed an association between corticosteroid response and increased synthesis of pulmonary surfactant in patients with the chronic fibrosing lung disease, cryptogenic fibrosing alveolitis (CFA) (synonym: idiopathic pulmonary fibrosis [IPF]). Patients with CFA, like those with ARDS but to a much lesser degree, have evidence of damage to the alveolar epithelium and endothelium, increased lung permeability and significant increases in BAL neutrophils. Moreover, we have found that a third of patients with CFA also show significant decreases in the surfactant phospholipid PG, and a quarter show significant increases in SM in BAL[20]. The PG and SM levels returned to normal in patients who responded to corticosteroid therapy. These observations suggest that disturbances of the pulmonary surfactant system may also contribute to the pathophysiology of CFA. Reductions in PG in CFA (IPF) have also been reported by Robinson et al[21] and in an experimental model of pulmonary fibrosis induced by bleomycin by Thrall et al[22]. The functional consequences of PG deficiency in adult lungs are unclear, but experimental models of acute lung injury show that this pattern of change in BAL phospholipid composition can occur in association with regeneration of type II alveolar epithelial cells after injury[23]. McCormack et al[24] have recently reported that SP-A levels are also significantly decreased in lavage samples from patients with CFA (IPF); and that those with the lowest pretreatment levels have an especially poor prognosis. These observations emphasise that there is a need for more extensive research to elucidate the contribution of surfactant deficiencies to the pathophysiology of chronic, as well as acute lung diseases.

Role in host defence

The focus of research on the functions of pulmonary surfactant has been mainly on its biophysical properties which regulate alveolar surface tension and prevent lung collapse. However, the pulmonary surfactant system also appears to play an important role in pulmonary immune defence mechanisms. Evidence from studies of BAL samples suggests that normal pulmonary surfactant promotes the natural 'non-specific' immune defence mechanisms which protect the air spaces against inhaled particles and microbes; but, by contrast, it suppresses the development of specific immune responses mediated by lymphocytes. Many studies have shown that lymphocytes from the alveolar spaces of normal lungs are less responsive to mitogenic and antigenic stimulation than their counterparts in the blood. Mechanisms appear to exist to down-regulate and avoid the development of lymphoproliferative responses to the many antigens we inhale each day from the environment. Presumably this is essential to prevent a constant state of immune hyperreactivity in the lungs which would be detrimental to life. The first evidence that pulmonary surfactant is immunosuppressive to lymphocytes came from the observation of Ansfield et al[25] that normal surfactant form canine lungs suppresses the proliferation of canine blood lymphocytes. Subsequently, work from our group showed that normal pulmonary surfactant from human, pig and rabbit, also suppresses human blood lymphocyte proliferation to mitogens and alloantigens[26]. The lipid fraction was highly immunosuppressive; and studies with purified phospholipids demonstrated that the major phospholipid components of surfactant PC and PG were highly immunosuppressive, but that the minor components SM and PE which can increase due to tissue damage, are highly immunostimulatory[27]. Thus, its is possible that changes in the surfactant system, seen in both acute and chronic lung diseases, might favour the development of pulmonary inflammation.

On the other hand, there is strong evidence that pulmonary surfactant plays an important role in enhancing 'non-specific' immune defence mechanisms in the alveoli. Surfactant lipids and SP-A have been shown to be chemotactic for alveolar macrophages[28,29] and SP-A can enhance the phagocytosis and killing of microorganisms by alveolar macrophages[30]. The surfactant system also aids non-specific mechanical clearance mechanisms within the lungs through its biophysical properties which promote clearance of cells and particles towards the mucociliary escalator at end expiration, due to the increasing film pressure exerted by the surface monolayer of phospholipids.

In conclusion, it is clear from this brief review that BAL is of considerable value to study the pulmonary surfactant system; in particular, to investigate alterations in lung diseases, the biological properties, and the prospects and rationale for surfactant therapy.

Acknowledgements

The author thanks the British Lung Foundation and the Clinical Research Committee of the National Heart and Lung Hospitals for their support of our studies of pulmonary surfactant in ARDS; and all the colleagues who have collaborated.

REFERENCES

1. Pulmonary Surfactant. Robertson B, van Goldie LMG, Battenburg JJ, editores. Amsterdam: Elsevier, 1985.

2. Hills BA. The Biology of Surfactant. Cambridge: Cambridge University Press, 1988.
3. Possmayer F. Pulmonary perspective: a proposed nomenclature for pulmonary surfactant associated proteins. Am Rev Respir Dis 1988; 138:990-988.
4. Wright JA, Wager RE, Hawgood S, Dobbs L & Clements JA. Surfactant apoprotein Mr = 26,000-36,000 enhances uptake of liposomes by type II cells. J Biol Chem 1987; 262:2.888-2-894.
5. Avery ME, Mead J. Surface properties in relation to atelectasis and hyaline membrane disease. Am J Dis Child 1959; 97:517-523.
6. Jobe A, Ikegami M. Surfactant for the treatment of respiratory distress syndrome. Am Rev Respir Dis 1987; 136:1.256-1.275.
7. Lewis JF, Jobe AH. Surfactant and the adult respiratory distress syndrome. Am Rev Respir Dis 1993; 147:218-233.
8. Hughes DA, Haslam PL. Effect of smoking on the lipid composition of lung lining fluid and relationship between immunostimulatory lipids, inflammatory cells and foamy macrophages in extrinsic allergic alveolitis. Eur Respir J 1990; 3:1.128-1.139.
9. Suzuki Y, Fujita Y, Kogishi K. Reconstitution of tubular myelin from syntheti lipis and proteins assoiated with pig pulmonary surfactant. Am Rev Respir Dis 1989; 140:75-81.
10. Takahashi A & Fujiwara T. Proteolipid in bovine lung surfactant: its role in surfactant function. Biochem Biophys Res Comm 1986; 135:527-532.
11. Ashbaugh DG, Bigelow DB, Petty TL, Levine BE. Acute respiratory distress in adults. Lancet 1967; 2:319-323.
12. Weiland JE, Davis WB, Holter JF. Lung neutrophils in the adult respiratory distress syndrome. Clinical and pathological significance. Am Rev Respir Dis 1986; 133:218-225.
13. Seeger W, Pison U, Buchhorn R, Obertacke U, Joka T. Surfactant abnormalities and adult respiratory failure. Lung 1990; 168 Suppl:891-902.
14. Haslam PL, Hughes DA, Macnaughton PD, Baker CS, Evans TW. Surfactant replacement therapy in patients with late stage adult respiratory distress syndrome. Lancet 1994. In press.
15. Gregory T, Longmore W, Moxley M, Whitsett J, Reed C, Fowler A et al. Surfactant chemical composition and biophysical activity in acute respiratory distress syndrome. J Clin Invest 1991; 88:1976-1981.
16. Metz C, Sibbald J. Anti-inflammatory therapy for acute lung injury. A review of animal and clinical studies. Chest 1991; 100:1110-1119.
17. Meduri GU, Belenchia JM, Estes RJ, Wunderink RG, Torky ME & Leeper Jnr KV. Fibroproliferative phase of ARDS. Clinical findings and effects of corticosteroids. Chest 1991; 100:943-952.
18. Braude S, Haslam PL, Hughes DA, Macnaughton P, Evans T. 'Chronic' adult respiratory distress syndrome. A role for corticosteroids? Critical Care Medicine 1992; in press.
19. Smith BT, Sabry K. Glucocorticoid-thyroid synergism in lung maturation: a mechanism involving epithelial-mesenchymal interaction. Proc Nat Acad Sci (Wash.) 1983; 80:1951-1954.
20. Hughes DA, Haslam PL. Changes in phosphatidylglycerol in bronchoalveolar lavage fluids from patients with cryptogenic fibrosing alveolitis. Chest 1989; 95:82-89.
21. Robinson PC, Watters LC, King TE, Jr., Mason RJ. Idiopathic pulmonary fibrosis: abnormalities in bronchoalveolar lavage phospholipids. Am Rev Respir Dis 1988; 137:585-591.
22. Thrall RS, Sendsen CL, Shannon TH, Kennedy CA, Fredrick DS, Grunze MF, Sulavik SB. Correlation of changes in pulmonary surfactant phospholipids with compliance in bleomycin-induced pulmonary fibrosis in the rat. Am Rev Respir Dis 1987; 136:113-118.
23. Liau DF, Barrett CR, Bell ALL & Ryan SF. Normal surface properties of phosphatidylglycerol-deficient surfactant from dog after acute lung injury. J Lipid Res 1985; 26:1338-1344.
24. McCormack FX, King Jr, TE, Voelker DR, Robinson PC, Mason RJ. Idiopathic pulmonary fibrosis. Abnormalities in the bronchoalveolar lavage content of surfactant protein A^{1-3}. Am Rev Respir Dis 1991; 144:160-166.
25. Ansfield MJ, Kaltreider HB, Benson BJ, Caldwell JL. Immunosuppressive activity of canine pulmonary surface active material. J Immunol 1979; 122:1062-1066.
26. Wilsher ML, Hughes DA, Haslam PL. Immunoregulatory properties of pulmonary surfactant: effect of lung lining fluid on proliferation of human blood lymphocytes. Thorax 1988; 43:354-359.
27. Wilsher ML, Hughes DA, Haslam PL. Immunoregulatory properties of pulmonary surfactant: influence of variations in the phospholipid profile. Clin Exp Immunol 1988; 73:117-122.
28. Schwartz LW, Christman CA. Lung lining material as a chemoattractant for alveolar macrophages. Chest 1979; 75:284-288.
29. Hoffman RM, Claypool WD, Katyal SL, Singh G, Rogers RM, Dauber JH. Augmentation of rat alveolar macrophage migration by surfactant protein. Am J Respir Dis 1987; 135:1358-1362.
30. van Iwaarden F, Welmers B, Verhoef J, Haagsman HP, van Golde LM. Pulmonary surfactant protein A enhances the host-defense mechanism of rat alveolar macrophages. Am J Respir Cell Mol Biol 1990; 2:91-98.